



## Modern Holstein-origin dairy cows within grassland-based systems partition more feed nitrogen into milk and excrete less in manure

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1    **Modern Holstein-origin dairy cows within grassland-based systems partition more feed**  
2    **nitrogen into milk and excrete less in manure**

3

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## 17 **ABSTRACT**

18 The objective was to determine whether modern Holstein-origin dairy cows, when managed  
19 within grassland-based systems, partitioned more feed nitrogen (N) into milk and excreted  
20 less in manure, in comparison to an earlier population of Holstein-origin dairy cows. Data  
21 used were collated from total diet digestibility studies undertaken in Northern Ireland from  
22 1990 to 2002 (old dataset, n = 538) and from 2005 to 2019 (new dataset, n = 476),  
23 respectively. An analysis of variance indicated that cows in the new dataset partitioned a  
24 significantly higher proportion of consumed N into milk and excreted a lower proportion in  
25 urine and total manure, compared to cows in the old dataset. A second analysis using the  
26 linear regression revealed that in comparison to the old dataset, the new dataset had a lower  
27 slope in the relationship between N intake and N excretion in urine or total manure, while a  
28 higher slope in the relationship between N intake and milk N output. A third analysis used the  
29 combined data from both datasets to examine if there was a relationship between  
30 experimental year and N utilization efficiency. Across the period from 1990 to 2019, urine  
31 N/N intake and manure N/N intake significantly decreased, while milk N/N intake increased.  
32 These results indicate that modern Holstein-origin dairy cows utilize consumed N more  
33 efficiently than earlier populations. Thus, N excretion is likely to be overestimated if models  
34 developed from the old data are used to predict N excretion for modern dairy herds.  
35 Therefore, the final part of analysis involved using the new dataset to develop prediction  
36 models for N excretion based on N intake and farm level data (milk yield, live weight and  
37 dietary N concentration). These updated models can be used to estimate N excretion from  
38 modern Holstein-origin dairy cows within grassland-based dairy systems.

39

40 **Keywords:** Grassland-based system, Holstein-origin cow, Manure nitrogen, Milk nitrogen,  
41 Prediction equation

42    **Abbreviations:**

43    AFBI, Agri-Food and Biosciences Institute; ANOVA, analysis of variance; CP, crude  
44    protein; DIM, days in milk; DN, diet nitrogen concentration; DM, dry matter; DMI, dry  
45    matter intake; ECMY, energy corrected milk yield; EU, European Union; FG, fresh grass;  
46    GS, grass silage; LW, live weight; ME, metabolizable energy; MS, maize silage; N, nitrogen;  
47    NDF, neutral detergent fiber; NI, nitrogen intake; RMSPE, root mean square prediction error;  
48    WCW, whole crop wheat silage;

## 49    **1. Introduction**

50    The loss of nitrogen (N) from livestock production systems can have a significant  
51    environmental impact (Tamminga, 1992; Yan *et al.*, 2006). For example, N losses to  
52    waterways can cause aquatic eutrophication, N emissions as nitrous oxide can lead to  
53    stratospheric ozone depletion and to global warming, while ammonia deposition on sensitive  
54    ecosystems can result in terrestrial eutrophication and soil acidification (Asman *et al.*, 1998;  
55    Hoekstra *et al.*, 2020). While dairy cows have a large requirement for N, with dairy cow diets  
56    typically containing crude protein (CP) in a range between 160 and 180 g/kg dry matter (DM)  
57    (Webster, 2020), much of feed N consumed is in excess of what animals can utilize, and is  
58    excreted in feces and urine (Huhtanen *et al.*, 2010; Powell *et al.*, 2017).

59

60    Urea comprises between 50% and 90% of total N in urine of high-producing dairy cows, and  
61    this urea is rapidly converted to ammonia, which is lost by volatilization when feces and  
62    urine mix (Bussink and Oenema, 1998; Hristov *et al.*, 2011). In Europe, approximately 75%  
63    of ammonia emitted to the atmosphere can be attributed to livestock production (Ding *et al.*,  
64    2020). Accurate predictions of the environmental impact of livestock production systems (for  
65    example, for estimating N volatilization, leaching, run-off, and emission), require N excretion  
66    from individual animals or groups of animals to be quantified with reasonable accuracy, and  
67    this is normally obtained from having an accurate estimate of N intakes and N utilization  
68    efficiency. A number of prediction models have been developed to predict N excretion in  
69    feces and urine from dairy cattle (e.g., Wilkerson *et al.*, 1997; Yan *et al.*, 2006; Reed *et al.*,  
70    2015).

71

72    The N utilization efficiency of dairy cows can be influenced by both dietary and animal  
73    factors, with diet quality (especially N concentration) and cow genetic merit likely to have a

74 significant effect on the efficiency with which dietary N is converted into milk N (Ferris *et*  
75 *al.*, 2018; O'Sullivan *et al.*, 2019). During the last 20 years dairy cow genotypes have  
76 improved considerably due to sire selection programs in most counties now focusing on both  
77 functional traits (e.g., fertility, health) and production traits (e.g., higher yielding cows with  
78 the ability to partition a greater proportion of nutrients into milk and less into body tissues)  
79 (Ferris *et al.*, 2018; Derno *et al.*, 2019). For example, the average annual milk production in  
80 the national dairy herd of Northern Ireland increased from 6,200 kg/yr in 2004 to 7,620 kg/yr  
81 in 2018 (Department of Agriculture, Environment and Rural Affairs, 2018). These  
82 improvements in cow genetic merit requires dairy producers to offer cows higher quality diets  
83 so as to meet their higher nutrient requirements. However, this may pose a great challenge for  
84 dairy producers in the European Union (EU) countries, due to the implementation of the  
85 Nitrate Directive program in the EU in 2000s that restricts application rates of organic and  
86 inorganic N to agricultural lands, forcing the dairy industry to adopt balanced diets with  
87 reduced N input. These factors can obviously influence the N utilization efficiency of dairy  
88 cow production. However, there is little information available to systematically evaluate if  
89 modern Holstein-origin dairy cows, managed within grassland-based dairy systems, can  
90 utilize N more efficiently than earlier populations of Holstein-origin dairy cows. Therefore,  
91 the present study used the analysis of variance (ANOVA) and linear regression techniques to  
92 examine if the N utilization efficiency of dairy cows differed within two dairy cow datasets  
93 which were collated from total diet digestibility studies undertaken at the Agri-Food and  
94 Biosciences Institute (AFBI) of Northern Ireland from 1990 to 2002, and from 2005 to 2019,  
95 respectively. The latter dataset was also used to develop prediction equations for N excretion  
96 for modern dairy cow production. The division of the year gap between the two datasets was  
97 due to the implementation of the EU's Nitrate Directive program in Northern Ireland in 2005-

98 2006. This program restricts application rates of N fertilizers to agricultural lands that  
99 consequently forces the dairy industry to reduce N input for dairy production.

100

## 101 **2. Materials and Methods**

### 102 *2.1. Animal, Diet and Digestibility Measurement*

103 Two N utilization datasets for lactating dairy cows were used in the present study, data within  
104 each having been collated from total diet digestibility studies undertaken at AFBI in Northern  
105 Ireland. The first dataset comprised data from experiments undertaken between 1990 and  
106 2002 (n = 538), while the second dataset comprised data from experiments undertaken  
107 between 2005 and 2019 (n = 476). Hereafter, these datasets are referred to as the ‘old dataset’  
108 and the ‘new dataset’, respectively. The new dataset was also used to develop prediction  
109 equations for N excretion for modern dairy cow production. The old dataset represents data  
110 collected prior to the implementation of the EU’s Nitrate Directive in Northern Ireland in the  
111 form of a Nitrates Action Program in 2005-2006.

112

113 The information on numbers of experiments, treatments, and cows, on cow genotypes, and  
114 forage types offered within each of the two datasets are presented in Table 1. Data on milk  
115 production, feed intake, N intake and outputs, and N utilization efficiency, within the 2  
116 datasets are presented in Table 2. Before commencing the digestibility trials, all cows were  
117 housed in free-stall cubicle accommodation and offered experimental diets *ad libitum* for at  
118 least 20 d. Thereafter, all cows were transferred to a metabolism unit for a further 8 d. During  
119 this time feed intake was recorded daily, while samples of forages and concentrates offered  
120 were taken daily and analyzed for chemical composition. Feces and urine were collected  
121 separately and sampled daily during the final 6 d in the metabolism unit to allow total ration  
122 digestibility to be determined. Details of feces and urine collection, feed sampling, and

123 methods used for analysis of feed, feces and urine samples were as described by Yan *et al.*  
124 (2006). Milk yields were recorded daily with milk samples taken during both morning  
125 (starting at 0500 h) and afternoon (starting at 1630 h) milking during the 8 d in metabolism  
126 units. Fat, protein and lactose concentrations of milk samples were analyzed using the  
127 methods described by Yan *et al.* (2006). Live weight (LW) was recorded on the first and last  
128 d in the metabolism unit. Animals had free access to water throughout the whole  
129 experimental period.

130

## 131 2.2. Statistical Analysis

132 Data analysis was conducted using Genstat 19<sup>th</sup> edition (VSN International, 2017). The two  
133 datasets (e.g., feed intake, milk production, N intake and output, and N utilization efficiency)  
134 were firstly compared using ANOVA, with the effects of animal [LW, milk yield, parity,  
135 days in milk (DIM), days in pregnancy] and dietary [forage proportion and concentrations of  
136 neutral detergent fiber (NDF), CP and metabolizable energy (ME)] factors removed, where  
137 appropriate. Linear regression analysis was then used to related total N intake, to N output in  
138 feces, urine or manure, with the objective to evaluate if there was significant difference in the  
139 slopes (with a common intercept) between the two datasets (old data vs. new data), or if there  
140 was any significant difference in the intercepts (with a common slope). The relationship  
141 between each response variable and each explanatory variable was fitted as a linear mixed  
142 model using the residual maximum likelihood (REML) commands. Diets and animals within  
143 experiments were fitted as random effects in all models, and the explanatory variable was as  
144 the fixed effect. Additional combinations of covariates, when appropriate, were also fitted as  
145 supplementary random effects for evaluation of N utilization efficiencies, which included  
146 milk yield, parity, DIM, days in pregnancy, dietary forage proportion, and dietary contents of  
147 NDF, CP and ME. The significance or otherwise of fixed effects was assessed by comparing



148 a Wald statistic against the appropriate F-distribution. If any of additional fixed effects was  
149 not significant ( $P > 0.05$ ), then it was removed from the analysis and the model was refitted.  
150 Several different models were fitted to each pair of response/explanatory variables in turn.  
151 First, a single line was fitted for all two datasets, and then two linear relationships using the  
152 two datasets (old vs. new datasets) were developed to compare the two slopes (with a  
153 common intercept) or the two intercepts (with a common slope). For the latter two models,  
154 pair-wise differences between different intercepts or slopes were also calculated if the main  
155 effect was significant using the Fisher's least significant difference test. Finally, an  
156 assessment of the goodness-of-fit of each model was made by calculating a pseudo  $R^2$   
157 (calculated in each case as the square of the correlation of the fitted values from the model  
158 with the observed values for the response variable). A third analysis involved examining if  
159 there was a linear relationship between experimental year and N partitioning rates for milk  
160 production and manure N excretion, using the combined data within the old and new datasets.  
161 Random effects were taken into account for each model, including experiment and animal  
162 (LW, milk yield, parity, DIM, days in pregnancy) and dietary (forage proportion and  
163 concentrations of NDF, CP and ME) factors.

164

165 Since the above comparisons demonstrated that the new dataset had a significantly higher N  
166 utilization efficiency than the old dataset, the new dataset was then used to develop a range of  
167 new models for predicting N excretion from 'modern' dairy herds. These new models (linear  
168 and multiple regression models) were developed, using the REML variance components  
169 analysis, to predict N excretion in feces, urine or total manure using N intake or a  
170 combination of LW, milk yield and dietary N concentration as explanatory variables.  
171 Random factors, including experiment, trial year, forage type, breed, parity and DIM, were  
172 fitted into each model with the objective of removing the effects of these random factors from

each relationship. These new equations were evaluated through an internal validation exercise, by dividing the whole new dataset ( $n = 476$ ) into two sub-datasets, i.e., two-thirds of data ( $n = 317$ ) vs. one-third of data ( $n = 159$ ). The selection was based on individual treatments/periods within each study, which ensures that each sub-dataset had a similar presentation of data variations as the whole dataset. The two-thirds of data were used to develop similar prediction equations to those developed using the whole dataset. These new prediction equations were then evaluated using the one-third of data. Prediction accuracy of relationships was examined using the root mean square prediction error (RMSPE), which is defined below (Equation a):

$$RMSPE = \sqrt{\frac{1}{n} \sum_{i=1}^n (P_i - A_i)^2} \quad [a]$$

Where  $P_i$  or  $A_i$  is the predicted or actual N output;  $n$  is the number of pairs of values of  $P_i$  and  $A_i$  compared.

### 3. Results

#### 3.1. Comparison of Cow Performance and N Utilization Data between the Old and New Datasets Using ANOVA

In comparison to the old dataset, cows in the new dataset had higher milk yield, energy-corrected milk yield (ECMY) and DIM, but lower LW ( $P < 0.001$ ; Table 2). Daily forage DM intake (DMI), concentrate DMI and total DMI were 0.7 kg, 1.1 kg and 1.8 kg higher ( $P < 0.001$ ), respectively, in the new compared to the old dataset, but diets offered in the new dataset had a lower forage proportion ( $P = 0.015$ ). Diets in the old dataset had a mean CP concentration of 0.011 kg/kg DM higher than those in the new dataset ( $P < 0.001$ ), while mean diet ME concentration was identical between the two datasets.

196

197 Cows in the new dataset had a greater N intake ( $P = 0.015$ ), and consequently higher ( $P <$   
198  $0.001$ ) feces N output, milk N output and retained N than those in the old dataset, while those  
199 in the old dataset had a higher ( $P < 0.001$ ) urine N output and manure N output. Nitrogen  
200 losses from urine and manure, when expressed as a proportion of N consumed, were lower ( $P$   
201  $< 0.001$ ) for cows in the new than the old dataset, but feces N, milk N and retained N as a  
202 proportion of N intake were higher ( $P < 0.001$ ) for those in the new dataset.

203

### 204 *3.2. Regression Analysis of N Utilization Data between the Old and New Datasets*

205 The linear regression technique was used to determine if there were differences in N  
206 utilization efficiency between the old and new dataset, through the comparison of slopes  
207 (with a common intercept) or intercepts (with a common slope) in each set of the linear  
208 relationship between N output and N intake. The results for comparison of slopes (with a  
209 common intercept) are presented in Table 3 and Figure 2. Feces N, urine N, manure N, milk  
210 N and retained N were each positively and significantly ( $P < 0.05$ ) related to N intake, with  
211  $R^2$  values ranging from 0.517 to 0.905 (Eq. [1a] to [5b]). With a common intercept, in  
212 comparison to the old dataset, the new dataset had a greater slope in the relationship of N  
213 intake with feces N ([1a] vs. [1b],  $P = 0.037$ ), milk N ([4a] vs. [4b],  $P < 0.001$ ) and retained  
214 N ([5a] vs. [5b],  $P = 0.009$ ), but a lower slope in relationship of N intake with urine N ([2a]  
215 vs. [2b],  $P < 0.001$ ) and manure N ([3a] vs. [3b],  $P < 0.001$ ). A similar result for comparison  
216 of intercepts (with a common slope) was also obtained (Table 4). With a common slope,  
217 intercepts derived from relationships of N intake with feces N ([6a] vs. [6b]), milk N ([9a] vs.  
218 [9b],  $P = 0.011$ ) and retained N ([10a] vs. [10b],  $P = 0.035$ ) were bigger in the new than old  
219 dataset, while the new dataset had a lower intercept in the relationship with urine N ([7a] vs.  
220 [7b],  $P < 0.001$ ) and manure N ([8a] vs. [8b],  $P < 0.001$ ).

221

### 222 *3.3. Relationships between Experimental Year and N Utilization Using the Combined Data*

223 The third evaluation was undertaken to examine if there was any relationship between  
224 experimental year and N utilization efficiency using the combined data from both old and  
225 new datasets. The results are presented in Table 5. The result revealed a negative relationship  
226 between experimental year and both urine N/N intake and manure N/N intake, and a positive  
227 relationship with milk N/N intake.

228

### 229 *3.4. Prediction Equations for N Excretion Developed Using the New Dataset*

230 Since the above evaluation indicates that ‘modern cows’ in the new dataset can utilize diet N  
231 more efficiently than cows in the old dataset, a range of updated prediction equations for N  
232 excretion for modern dairy production were developed using the new dataset (Table 6). The  
233 relationships between N excretion and N intake are also presented in Fig. 1. All relationships  
234 were significant ( $P < 0.001$ ), and each predictor had a significant effect on the relationship ( $P$   
235  $< 0.001$ ). Nitrogen intake is a good predictor of N excretion in urine and manure ( $R^2 = 0.783$   
236 and  $0.833$ , respectively), although the  $R^2$  value ( $0.684$ ) for prediction of feces N output is  
237 relatively low. As N intake data are not always available, especially in commercial farms,  
238 farm-level data (ECMY, LW and diet N concentration) were also used to develop prediction  
239 equations. The  $R^2$  values were  $0.774$  and  $0.779$ , respectively, for prediction of N excretion in  
240 urine and total manure, although the  $R^2$  value for prediction of feces N output is relatively  
241 low ( $R^2 = 0.593$ ).

242

243 These updated equations were evaluated through an internal validation exercise (Table 7). All  
244 equations produced a mean predicted value that is close to the mean actual data in the  
245 prediction of N excretions in feces, urine and total manure. All predictions had a relatively

246 small RMSPE. In addition, farm level data (ECMY, LW and diet N concentration) can be  
247 used to predict feces N and urine N outputs with a similar accuracy to those predicted using  
248 N intake, in terms of RSMPE and SE values, although prediction of manure N output had  
249 marginally higher RSMPE and SE values when predicted using farm level data.

250

## 251 **4. Discussion**

252 The present study was designed to evaluate the effects of dietary N inputs, and genetic  
253 improvements within the Holstein dairy cow population, on N utilization efficiency for milk  
254 production and N excretion rate in manure. Within the EU, pressure to improve N utilization  
255 efficiency has been driven in part by the EU Nitrates Directive which was designed to reduce  
256 N losses of agricultural origin to waterways (EU, 1991), as well as concerns about global  
257 warming, and the impact of ammonia on sensitive habitats. The two datasets used in the  
258 present study were obtained from studies undertaken at AFBI in Northern Ireland, and  
259 involved dairy cows of the Holstein breed (including Holstein crossbreds), offered  
260 predominantly grass silage based diets. However, grassland-based systems in Northern  
261 Ireland have much in common with systems adopted in many other grassland regions of the  
262 world, including western parts of the United Kingdom, Republic of Ireland and much of  
263 Northern Europe. In addition, the AFBI herd is bred entirely by artificial insemination, using  
264 high genetic merit sires sourced globally, and as a result is genetically similar to many high  
265 producing Holstein herds throughout the world. Thus the outcomes of the present study has  
266 applicability beyond Northern Ireland.

267

### 268 *4.1. Nitrogen Utilization Efficiency*

269 The present study indicates that modern dairy cows utilize feed N more efficiently than  
270 previous dairy cow populations (over 15 years ago). In comparison with the old dataset, cows

271 in the new dataset utilized a higher proportion of N intake for milk production, and excreted a  
272 lower proportion of N intake in urine and total manure. A linear regression between  
273 experimental year and N utilization efficiency data involving the combined old and new  
274 datasets demonstrated a significant reduction in the ratios of urine N/N intake, and manure  
275 N/N intake, and a significant increase in milk N/N intake over the last two decades. These  
276 results imply that, with lower diet N inputs, modern dairy herds can maintain a similar milk  
277 production and excrete less N in manure, when compared to those over 15 years ago. In  
278 addition, it is worth noting that cows in the new dataset had a considerably lower proportion  
279 of urine N over N intake. The reduction in urinary N excretion is likely to help reduce  
280 ammonia loss to the environment, with potentially beneficial effects on air quality and  
281 biodiversity in sensitive habitats.

282

283 Many dietary, animal and management factors can influence N utilization efficiency of dairy  
284 cows (ARC, 1980). Perhaps, the most important factor is to feed dairy cows balanced diets  
285 which synchronize the supply of degradable N and fermentable energy to optimize rumen  
286 microbial activity and milk production. The oversupply of degradable N can cause the  
287 excessive ammonia in the rumen to be absorbed into bloodstream and excreted in urine as  
288 urea (Burgos *et al.*, 2010). In the present study, the higher N utilization efficiency derived  
289 from the new vs. old dataset could be attributed to lower dietary CP concentrations in the new  
290 dataset (0.174 vs. 0.183 kg/kg DM,  $P < 0.001$ ), because dietary ME concentration in the two  
291 datasets was identical, although the new dataset had a slightly lower dietary forage proportion  
292 (0.554 vs. 0.579 kg/kg DM,  $P = 0.006$ ). The statistical analysis of the present two datasets  
293 found that the new dataset had lower ratios of urine N and manure N over N intake, although  
294 fecal N/N intake was higher in the new dataset. The linear regression analysis using the  
295 combined data of the present new and old datasets also found a similar result (Fig. 3).

296 Increasing dietary CP concentrations significantly increased N excretion rates in urine and  
297 total manure but decreased fecal N output rate ( $P < 0.001$ ). Although there is no comparable  
298 publication using data collated from a range individual total diet studies undertaken at  
299 different periods of years, there are a range of individual studies of dairy cows which  
300 obtained similar results to the present study. For example, Broderick (2003) found a reduced  
301 urine N (from 0.362 to 0.238 g/g) but increased fecal N (from 0.296 to 0.403 g/g) as  
302 proportion of N intake in lactating dairy cows offered diets containing dietary CP varied from  
303 0.135 to 0.194 kg/kg DM. Hristov *et al.* (2004) reported that increased dietary CP  
304 concentration resulted in decreased efficiency of conversion of dietary N into milk protein  
305 and less efficient use of ruminal ammonia N for milk protein syntheses, with excess largely  
306 lost through urinary N excretion. Increasing dietary CP concentrations were found to increase  
307 dilution of metabolic fecal N, and increase N digestibility, and also increase urinary N  
308 excretion (Marini and Van Amburgh, 2005). In addition, reduced dietary N/ME and CP  
309 concentration have been reported to improve N utilization efficiency with less N excreted in  
310 urine of dry cows (Stergiadis *et al.*, 2015a). The reduction of N excretion in urine implies less  
311 ammonia emissions from dairy production systems, as urinary urea can be rapidly hydrolyzed  
312 to ammonia by the urease enzyme in less than 24 h in grazing (Petersen *et al.*, 1998) and  
313 confined animals (James *et al.*, 1999). Frank *et al.* (2002) found, on average, a 2/3 decrease in  
314 ammonia release to air from manure of dairy cows offered diets containing CP of 0.140 vs.  
315 0.190 kg/kg DM without significant effect on milk production. These findings, together with  
316 the present result, indicate that manipulating dietary CP concentration could be an effective  
317 strategy to improve N utilization efficiency and reduce N excretion and ammonia emissions  
318 in dairy cow production.  
319

320 The increase in N utilization efficiency observed with the modern dairy cows in the new  
321 dataset may also be due to the continuous improvement in cow genetic merit over time.  
322 Indeed, cow genetic merit (expressed as £Profitable Lifetime Index, 2018 base year) of  
323 Holstein cows in AFBI dairy herd, from which dairy cows used in experiments of the present  
324 study were selected, improved by £23.3 per year from 1993 to 2017 (Fig 4). Profitable  
325 Lifetime Index, a composite financial index used within the UK, includes milk production  
326 and a number of other functional traits including health, fertility and longevity. Selecting  
327 sires on the basis of £ Profitable Lifetime Index has also improved the milk production  
328 potential of the herd, resulting in cows with higher nutrient requirements to meet their greater  
329 energy demand for milk production. Increasing the level of feeding can increase the rumen  
330 outflow rate, and leave less time available for rumen microbial activity, thus reducing protein  
331 degradability in the rumen and consequently N excretion in urine. Indeed, in a study to  
332 evaluate the effect of cow genetic merit on the production efficiency, Ferris *et al.* (1999)  
333 found that high merit cows had higher DM intake and milk production, but lower urine N  
334 output as a proportion of N intake, when compared with low merit cows. Yan *et al.* (2006), in  
335 a meta-analysis of a large digestibility dataset, reported a reduced ratio of manure N/N intake  
336 with increasing milk yield from <15, 15-30 to >30 kg/d. Cheng *et al.* (2014) reported a  
337 positive relationship between N utilization efficiency and cow's genetic merits when fed with  
338 freshly-cut perennial ryegrass. On the other hand, high genetic merit cows were found to have  
339 the ability to partition more nutrients into milk and less into body tissue than medium or low  
340 genetic merit cows (Agnew and Yan, 2000; Mehtiö *et al.*, 2018). Gordon *et al.* (1995)  
341 demonstrated that high genetic merit cows produced 6.60 and 8.25 kg/d more milk, and  
342 partitioned 13% and 8% more consumed N into milk, respectively, when compared with  
343 medium and low genetic merit cows. These results indicate that high genetic merit cows  
344 utilize feed N for milk production more efficiently than lower genetic merit cows.



345 Consequently, modern dairy cows can excrete less N in feces and urine, per kg of standard  
346 milk.

347

#### 348 4.2. Prediction Equations for N excretion

349 The present study revealed that modern dairy cows had a higher N utilization efficiency than  
350 previous populations over 15 years ago. Thus using equations developed using data from  
351 studies undertaken over 15 years ago may over-predict N excretions in feces and urine for  
352 modern dairy cows. Therefore, two sets of updated prediction equations for fecal N, urinary  
353 N and manure N were developed using the new dataset in the present study. One set of  
354 equations is based on N intake and the other based on farm level data (LW, milk yield and  
355 diet N concentration). Nitrogen intake has been found to be a better predictor of urine N  
356 (Reed *et al.*, 2015) and manure N output (Yan *et al.*, 2006) than farm level data (e.g., LW or  
357 LW and milk yield) in both dairy cows and beef cattle (Dong *et al.*, 2014; Jiao *et al.*, 2014;  
358 Reed *et al.*, 2015). In the present study, using N intake as a single predictor for fecal N, urine  
359 N and manure N output produced responses with relatively high  $R^2$  values (0.684, 0.783 and  
360 0.833, respectively). These values are comparable to those in young Holstein steer and heifer  
361 offered grass silage (0.75, 0.73 and 0.86, respectively; Jiao *et al.*, 2014), but higher than those  
362 in non-pregnant cows offered fresh grass (0.50, 0.61 and 0.60, respectively; Stergiadis *et al.*,  
363 2015b), and that (0.78) of relationship between N intake and manure N output (Kebreab *et al.*,  
364 2001) using a small dataset of lactating dairy cows. Since information on N intake is not  
365 always available, especially on commercial farms, a range of prediction equations using farm  
366 level data (LW, milk yield and diet N concentration) were also developed in the present  
367 study. Although the  $R^2$  value (0.593) for prediction of feces N output was relatively low, the  
368  $R^2$  values for prediction of urine N (0.774) and manure N (0.779) are comparable to those  
369 derived in the current study using N intake as the predictor. The present internal validation

370 also demonstrated that using these farm level data could produce a relatively accurate  
371 prediction of N excretion in feces, urine and total manure, when compared with those  
372 predicted using N intake. These equations provide a useful tool to estimate N excretion in  
373 feces and urine from Holstein-origin cows in commercial grassland-based dairy systems.

374

## 375 **5. Conclusion**

376 The present study showed that the modern Holstein-origin dairy cows managed within  
377 grassland-based systems utilized consumed diet N more efficiently, partitioning more  
378 consumed N into milk and less into urine and total manure, than earlier Holstein populations.  
379 The increase in N utilization efficiency not only improves the economical return to dairy  
380 producers, but also reduces N losses to the environment as nitrates, ammonia and nitrous  
381 oxide. In addition, the present study developed a range of prediction equations for manure N  
382 excretion using data collated from modern dairy cows, which provide a useful tool for the  
383 Holstein-origin dairy producers to mitigate N excretion under grassland-based farming  
384 conditions.

385

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389

## 390 **Declaration of Competing Interest**

391 The authors declare that there are no conflicts of interest.

392

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509 **Figures:**

510

511 **Figure 1.** Relationships between N intake and N excretion using data of dairy cows collated  
512 from experiments undertaken at AFBI from 1990 to 2002 (old dataset, A) and from 2005 to  
513 2019 (new dataset, B)

514

515 **Figure 2.** The comparison of N utilization efficiencies of dairy cows using data obtained  
516 between 1990-2002 (old dataset, dashed line) and 2005-2019 (new dataset, solid line) and the  
517 linear regression of N intake against N excretion in feces (A), urine (B) and total manure (C)

518

519 **Figure 3.** The relationships between diet crude protein (CP) concentration and N excretion  
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522

523 **Figure 4.** The improvement in the profitable lifetime index (base year - 2018) of Holstein  
524 dairy herd in the research farm of AFBI from 1993 to 2017



525 **Tables:**

526

527 **Table 1.** Information on experiment, treatment, cow breed and forage types in the old and  
528 new datasets of dairy cows used in the present study

529

530 **Table 2.** The ANOVA comparison of AFBI dairy cow digestibility variables using data  
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532

533 **Table 3.** The linear regression analysis (with common intercepts) of N utilization efficiencies  
534 of dairy cows using data obtained between 1990 and 2002 (old dataset) and 2005-2019 (new  
535 dataset)

536

537 **Table 4.** The linear regression analysis (with common slopes) of N utilization efficiencies of  
538 dairy cows using data obtained between 1990-2002 (old dataset) and 2005-2019 (new  
539 dataset)

540

541 **Table 5.** Relationships between experimental year and N utilization using the combined data  
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543

544 **Table 6.** Prediction of N output of dairy cows using total diet digestibility data (n = 476)  
545 collated from AFBI experiments undertaken from 2005 to 2019

546

547 **Table 7.** Internal validation – evaluation of prediction accuracy for N output of dairy cows  
548 using one third of the present data and equations developed from two thirds of the present  
549 data (data collated from studies undertaken at AFBI from 2005 to 2019)

**Table 1**[Click here to download Table: Table 1.doc](#)**Table 1.** Information on experiment, treatment, cow breed and forage types in the old and new datasets of dairy cows used in the present study

	Old dataset	New dataset
Years of experiments	1990-2002	2005-2019
Number of experiments	25	14
Number of treatments	134	74
Number of individual cow data	538	476
Cow breeds		
Holstein-Friesian	509	357
Others <sup>1</sup>	29	119
Forage types <sup>2</sup>	GS, FG	GS, MS, WCW

<sup>1</sup> Including Holstein crossbreds, Norwegian and Swedish Red.<sup>2</sup> GS = grass silage, FG = fresh grass, MS = maize silage, WCW = whole crop wheat silage

**Table 2**[Click here to download Table: Table 2.doc](#)

**Table 2.** The ANOVA comparison of AFBI dairy cow digestibility variables using data obtained between 1990 and 2002 (old dataset) and 2005-2019 (new dataset)

	Old dataset	New dataset	SED <sup>1</sup>	<i>P</i> -value
Number of cows	538	476	-	-
Animal data				
Lactation number	2.9	2.5	0.11	<0.001
Days in milk	154	170	4.7	<0.001
Live weight, kg	565	550	4.5	<0.001
Milk yield, kg/d	21.3	23.6	0.46	<0.001
Energy-corrected milk yield, kg/d	21.7	24.0	0.44	<0.001
Feed intake and composition <sup>2</sup>				
Forage DMI, kg/d	9.4	10.0	0.19	<0.001
Concentrate DMI, kg/d	7.1	8.2	0.22	<0.001
Total DMI, kg/d	16.4	18.2	0.20	<0.001
Forage proportion, kg/kg DM	0.585	0.554	0.0112	0.006
Diet CP concentration, kg/kg DM	0.183	0.174	0.0017	<0.001
Diet ME concentration, MJ/kg DM	12.1	12.1	0.06	0.96
N intake and output, g/d				
N intake	484	506	8.0	0.006
Feces N output	141	159	2.3	<0.001
Urine N output	208	178	4.4	<0.001
Manure N output	349	337	6.0	0.045
Milk N output	108	127	2.3	<0.001
Retained N	27	42	2.6	<0.001
N utilization efficiency				
Feces N/N intake	0.296	0.321	0.0034	<0.001
Urine N/N intake	0.428	0.348	0.0051	<0.001
Manure N/N intake	0.723	0.669	0.0047	<0.001
Milk N/N intake	0.226	0.252	0.0035	<0.001
Retained N/N intake	0.050	0.079	0.0051	<0.001

<sup>1</sup>Standard error of the difference.

<sup>2</sup>DMI = dry matter intake, DM = dry matter, CP = crude protein, ME = metabolizable energy

**Table 3**[Click here to download Table: Table 3.doc](#)

**Table 3.** The linear regression analysis (with common intercepts) of N utilization efficiencies of dairy cows using data obtained between 1990 and 2002 (old dataset) and 2005-2019 (new dataset)

		Equation <sup>1</sup>		R <sup>2</sup>	P-value	Eq. No
	Variable	Slope	Intercept			
Old dataset	Feces N =	0.270 <sub>(0.010)</sub> N intake	+ 12.0 <sub>(8.5)</sub>	0.816	0.037	1a
New dataset		0.285 <sub>(0.009)</sub> N intake				1b
Old dataset	Urine N =	0.407 <sub>(0.018)</sub> N intake	+ 11.7 <sub>(12.6)</sub>	0.832	< 0.001	2a
New dataset		0.333 <sub>(0.017)</sub> N intake				2b
Old dataset	Manure N =	0.673 <sub>(0.015)</sub> N intake	+ 25.7 <sub>(10.6)</sub>	0.905	< 0.001	3a
New dataset		0.614 <sub>(0.014)</sub> N intake				3b
Old dataset	Milk N =	0.102 <sub>(0.0077)</sub> N intake	+ 61.0 <sub>(12.6)</sub>	0.884	< 0.001	4a
New dataset		0.128 <sub>(0.0077)</sub> N intake				4b
Old dataset	Retained N =	0.221 <sub>(0.0141)</sub> N intake	- 83.2 <sub>(13.7)</sub>	0.517	0.009	5a
New dataset		0.250 <sub>(0.0146)</sub> N intake				5b

<sup>1</sup>Values in subscript parentheses are SE.

**Table 4**[Click here to download Table: Table 4.doc](#)

**Table 4.** The linear regression analysis (with common slopes) of N utilization efficiencies of dairy cows using data obtained between 1990-2002 (old dataset) and 2005-2019 (new dataset)

		Equation <sup>1</sup>		R <sup>2</sup>	P-value	Eq. No
	Variable	Slope	Intercept			
Old dataset	Feces N =	0.275 <sub>(0.009)</sub> N intake	+ 8.9 <sub>(8.50)</sub>	0.816	0.035	6a
New dataset			+ 18.3 <sub>(9.10)</sub>			6b
Old dataset	Urine N =	0.380 <sub>(0.017)</sub> N intake	+ 22.9 <sub>(12.7)</sub>	0.828	<0.001	7a
New dataset			- 9.5 <sub>(13.9)</sub>			7b
Old dataset	Manure N =	0.656 <sub>(0.014)</sub> N intake	+ 31.9 <sub>(10.9)</sub>	0.904	<0.001	8a
New dataset			+ 7.70 <sub>(11.8)</sub>			8b
Old dataset	Milk N =	0.012 <sub>(0.0073)</sub> N intake	+ 57.7 <sub>(12.7)</sub>	0.882	0.011	9a
New dataset			+ 66.2 <sub>(12.9)</sub>			9b
Old dataset	Retained N =	0.233 <sub>(0.0133)</sub> N intake	- 88.6 <sub>(13.8)</sub>	0.516	0.035	10a
New dataset			- 74.8 <sub>(14.3)</sub>			10b

<sup>1</sup>Values in subscript parentheses are SE.

**Table 5**[Click here to download Table: Table 5.doc](#)**Table 5.** Relationships between experimental year and N utilization using the combined data (from 1990 to 2019, with 1990 defined as year 1 and 2019 as year 30)

Variable	Equation <sup>1</sup>		R <sup>2</sup>	P-value	Eq. No
	Slope	Intercept			
Feces N/N intake	0.0010 <sub>(0.0002)</sub> EY	+ 0.294 <sub>(0.0033)</sub>	0.116	0.131	11
Urine N/N intake	- 0.0043 <sub>(0.0004)</sub> EY	+ 0.449 <sub>(0.0050)</sub>	0.419	0.001	12
Manure N/N intake	- 0.0032 <sub>(0.0003)</sub> EY	+ 0.743 <sub>(0.0046)</sub>	0.451	<0.001	13
Milk N/N intake	0.0021 <sub>(0.0002)</sub> EY	+ 0.213 <sub>(0.0033)</sub>	0.238	0.025	14

<sup>1</sup>Values in subscript parentheses are SE; EY denotes experimental year.

**Table 6**[Click here to download Table: Table 6.doc](#)

**Table 6.** Prediction of N output of dairy cows using total diet digestibility data (n = 476) collated from AFBI experiments undertaken from 2005 to 2019

Equations <sup>1</sup>	R <sup>2</sup>	Eq. No
Fecal N output (g/d) =		
$0.226_{(0.012)} \text{ NI} + 47.0_{(12.8)}$	0.684	15a
$0.091_{(0.022)} \text{ LW} + 2.64_{(0.23)} \text{ ECMY} + 1.64_{(0.40)} \text{ DN} + 1.2_{(17.3)}$	0.593	15b
Urine N output (g/d) =		
$0.366_{(0.018)} \text{ NI} - 10.1_{(17.9)}$	0.783	16a
$0.207_{(0.029)} \text{ LW} + 1.15_{(0.34)} \text{ ECMY} + 9.27_{(0.62)} \text{ DN} - 212.3_{(34.2)}$	0.774	16b
Manure N output (g/d) =		
$0.594_{(0.019)} \text{ NI} + 36.7_{(12.4)}$	0.833	17a
$0.665_{(0.018)} \text{ NI}$	0.833	17b
$0.277_{(0.040)} \text{ LW} + 3.68_{(0.45)} \text{ ECMY} + 11.32_{(0.81)} \text{ DN} - 206.9_{(42.5)}$	0.779	17c

<sup>1</sup>Values in subscript parentheses are SE. DN = diet N concentration, g/kg DM; ECMY = energy corrected milk yield, kg/d; LW = live weight, kg; NI = N intake, g/d

**Table 7.** Internal validation – evaluation of prediction accuracy for N output of dairy cows using one third of the present data and equations developed from two thirds of the present data (data collated from studies undertaken at AFBI from 2005 to 2019)

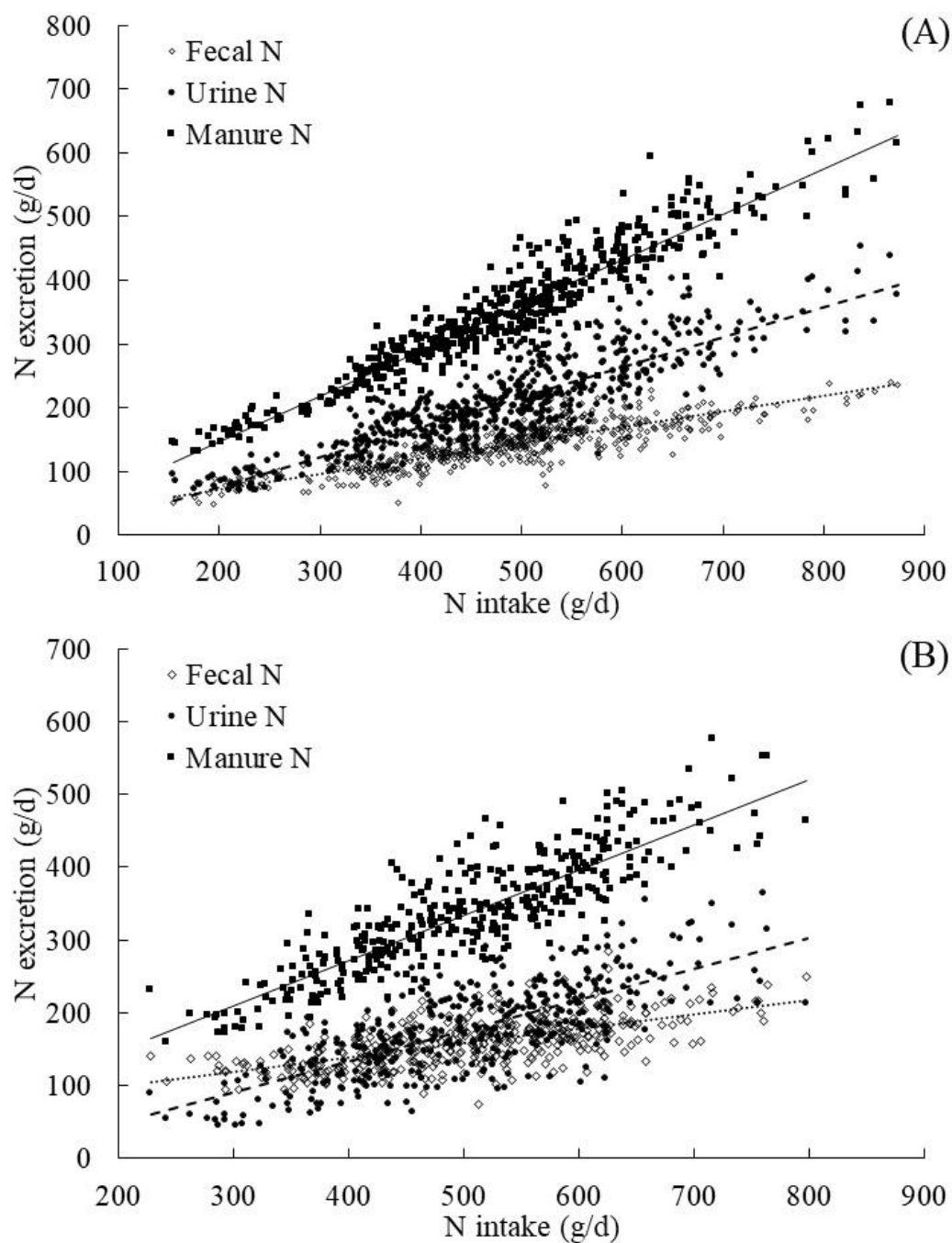
Predictors <sup>1</sup>	N output, g/d					Predicted – actual N output, g/d			
	Predicted	Actual	R <sup>2</sup>	RMSPE	SE	Mean	SD	Minimum	Maximum
N intake	161	159	0.48	2.05	17.4	2.44	23.3	-67.1	65.2
LW+ECMY+DN	159	159	0.40	2.20	17.8	0.17	25.1	-71.7	54.1
			Prediction of urine N output, g/d						
N intake	175	179	0.49	3.87	28.1	-4.76	43.9	-124	93.6
LW+ECMY+DN	186	179	0.56	3.65	27.3	6.97	41.0	-116	89.4
			Prediction of manure N output, g/d						
N intake	337	338	0.72	3.61	34.0	-1.52	41.1	-120	96.7
LW+ECMY+DN	347	338	0.52	4.72	38.7	8.71	53.1	-137	128

<sup>1</sup>DN = diet N concentration, g/kg DM, ECMY = energy corrected milk yield, kg/d, LW = live weight, kg, RMSPE = root mean square prediction error



**Figure 1**

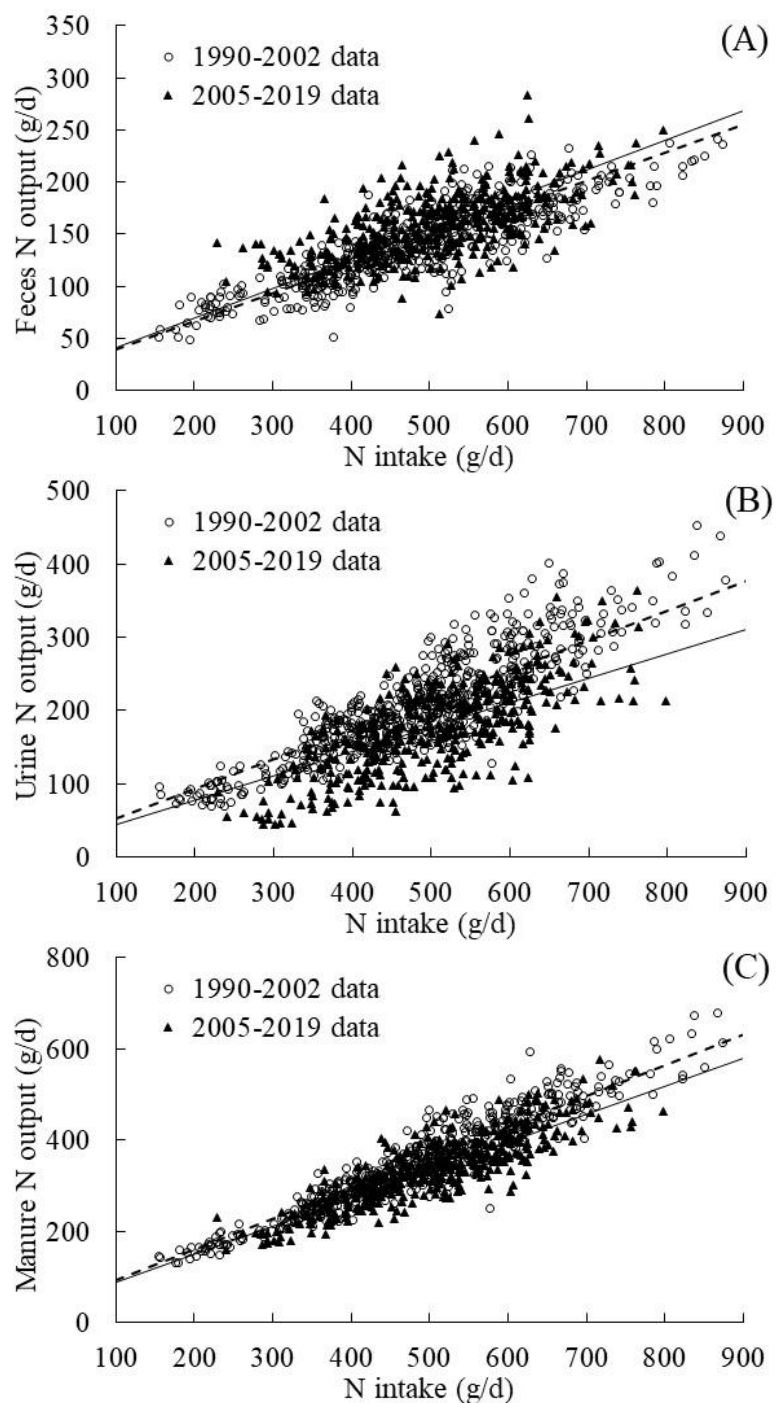
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**Figure 1.** Relationships between N intake and N excretion using data of dairy cows collated from experiments undertaken at AFBI from 1990 to 2002 (old dataset, A) and from 2005 to 2019 (new dataset, B)

**Figure 2**

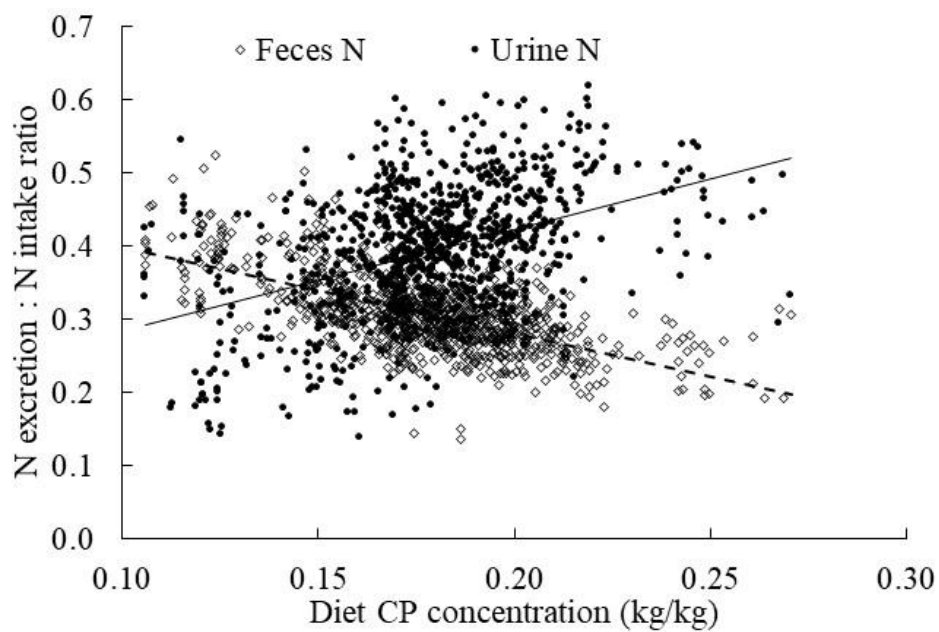
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**Figure 2.** The comparison of N utilization efficiencies of dairy cows using data obtained between 1990-2002 (old dataset, dashed line) and 2005-2019 (new dataset, solid line) and the linear regression of N intake against N excretion in feces (A), urine (B) and total manure (C)

**Figure 3**

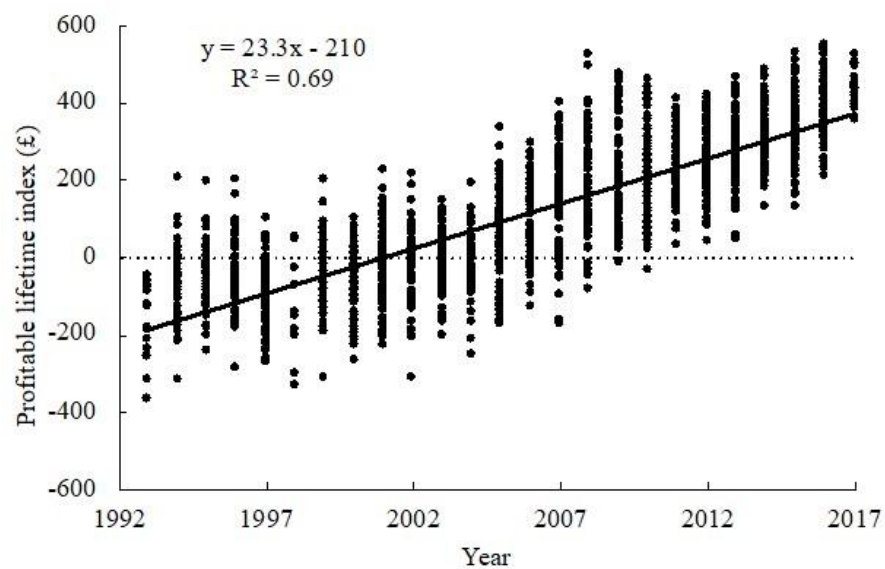
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**Figure 3.** The relationships between diet crude protein (CP) concentration and N excretion ratios in feces (dashed line) and urine (solid line) using the combined data of old and new datasets in the present study

**Figure 4**

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**Figure 4.** The improvement in the profitable lifetime index (base year - 2018) of Holstein dairy herd in the research farm of AFBI from 1993 to 2017